BISPECIFIC ANTIGEN-BINDING CONSTRUCTS TARGETING HER2

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application is a continuation of U.S. application Ser. No. 15/036,176, filed May 12, 2016 which was a 371 National Stage of International Application No. PCT/CA2014/051140, filed Nov. 27, 2014, which claims the benefit of U.S. Provisional Application No. 61/910,026, filed Nov. 27, 2013; U.S. Provisional Application No. 62/000,908, filed May 20, 2014; and U.S. Provisional Application No. 62/009,125, filed Jun. 6, 2014, which are all hereby incorporated in its entirety by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which will be submitted via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on Feb. 24, 2021, is named ZWI-017C2_Sequence_Listing.txt, and is 275,153 bytes in size.

BACKGROUND

[0003] The majority of current marketed antibody therapeutics are bivalent monospecific antibodies optimized and selected for high affinity binding and avidity conferred by the two antigen binding domains. Defucosylation or enhancement of FcgR binding by mutagenesis have been employed to render antibodies more efficacious via antibody Fc dependent cell cytotoxicity mechanisms. Afucyosylated antibodies or antibodies with enhanced FcgR binding still suffer from incomplete therapeutic efficacy in clinical testing and marketed drug status has yet to be achieved for any of these antibodies.

[0004] Therapeutic antibodies would ideally possess certain minimal characteristics, including target specificity, biostability, bioavailability and biodistribution following administration to a subject patient, and sufficient target binding affinity and high target occupancy to maximize antibody dependent therapeutic effects. Typically therapeutic antibodies are monospecific. Monospecific targeting however does not address other target epitopes that may be relevant in signaling and disease pathogenesis, allowing for drug resistance and escape mechanism. Some of the current therapeutic paradigms call for the use of combination of two therapeutic monospecific antibodies targeting two different epitopes of the same target antigen. One example is the use of a combination of Trastuzumab and Pertuzumab, both targeting the Her2 receptor protein on the surface of some cancer cells. Therapeutic antibodies targeting HER2 are disclosed in WO 2012/143523 to GenMab and WO 2009/154651 to Genentech. Antibodies are also described in WO 2009/068625 and WO 2009/068631.

[0005] Co-owned patent applications PCT/CA2011/001238, filed Nov. 4, 2011, PCT/CA2012/050780, filed Nov. 2, 2012, PCT/CA2013/00471, filed May 10, 2013, and PCT/CA2013/050358, filed May 8, 2013 describe therapeutic antibodies. Each is hereby incorporated by reference in their entirety for all purposes.

SUMMARY OF THE INVENTION

[0006] Described herein are bivalent antigen binding constructs that binding HER2. The antigen binding constructs comprise a first antigen binding polypeptide construct which monovalently and specifically binds a HER2 (human epidermal growth factor receptor 2) ECD2 (extracellular domain 2) antigen on a HER2-expressing cell and a second antigen-binding polypeptide construct which monovalently and specifically binds a HER2 ECD4 (extracellular domain 4) antigen on a HER2-expressing cell, wherein at least one of the ECD2- or the ECD4-binding polypeptide constructs is an scFv. In certain embodiments, the ECD2-binding polypeptide construct is an scFv, and the ECD2-binding polypeptide construct is a Fab. In certain embodiments, the ECD2-binding polypeptide construct is a Fab and the ECD4 binding polypeptide construct is an scFv. In some embodiments, both the ECD2- and ECD4-binding polypeptide constructs are scFvs. In some embodiments, the antigen binding constructs have a dimeric Fc comprising a CH3 sequence. In some embodiments, the Fc is a heterodimer having one or more modifications in the CH3 sequence that promote the formation of a heterodimer with stability comparable to a wild-type homodimeric Fc. In some embodiments, the heterodimeric CH3 sequence has a melting temperature (Tm) of 68° C. or higher. Also described are nucleic acids encoding antigen binding constructs, and vectors and cells. Also described are methods of treating a disorder, e.g., cancer, using the antigen binding constructs described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIGS. **1**A-**1**F depict structures of biparatopic antibodies. FIG. **1**A depicts the structure of a biparatopic antibody in a Fab-Fab format. FIGS. **1**B to **1**E depict the structure of possible versions of a biparatopic antibody in an scFv-Fab format. In FIG. **1**B, antigen-binding domain 1 is an scFv, fused to Chain A, while antigen-binding domain 2 is a Fab, fused to Chain B. In FIG. **1**C, antigen-binding domain 1 is a Fab, fused to Chain A, while antigen-binding domain 2 is an scFv, fused to Chain B. In FIG. **1**D, antigen-binding domain 2 is a Fab, fused to Chain A, while antigen-binding domain 1 is an scFv, fused to Chain B. In FIG. **1**E, antigen-binding domain 2 is an scFv, fused to Chain A, while antigen-binding domain 1 is a Fab, fused to Chain B. In FIG. **1**F, both antigen-binding domains are scFvs.

[0008] FIGS. **2**A-**2**C depict the characterization of expression and purification of exemplary anti-HER2 biparatopic antibodies. FIG. **2**A and FIG. **2**B depict the SEC chromatograph of the protein A purified antibody, and non-reducing SDS-PAGE analysis of 10 L expression and purification of v5019. FIG. **2**C depicts the SDS-PAGE analysis of a 25 L expression and purification of v10000.

[0009] FIG. **3**A and FIG. **3**B depict the results of UPLC-SEC analysis of exemplary anti-HER2 biparatopic antibodies purified by protein A and SEC. FIG. **3**A shows the results for v5019, where the upper panel shows the results of the purification and the lower panel shows the same result with an expanded scale for the y-axis. A summary of the data obtained is provided below the UPLC-SEC results. FIG. **3**B shows the results for v10000.

[0010] FIG. **4**A and FIG. **4**B depict LCMS analysis of the heterodimer purity of exemplary anti-HER2 biparatopic antibodies. FIG. **4**A depicts results from LC-MS analysis of the pooled SEC fractions of v5019. FIG. **4**B depicts the results from LC-MS analysis of the pooled protein A fractions of v10000.